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Supplemental Data

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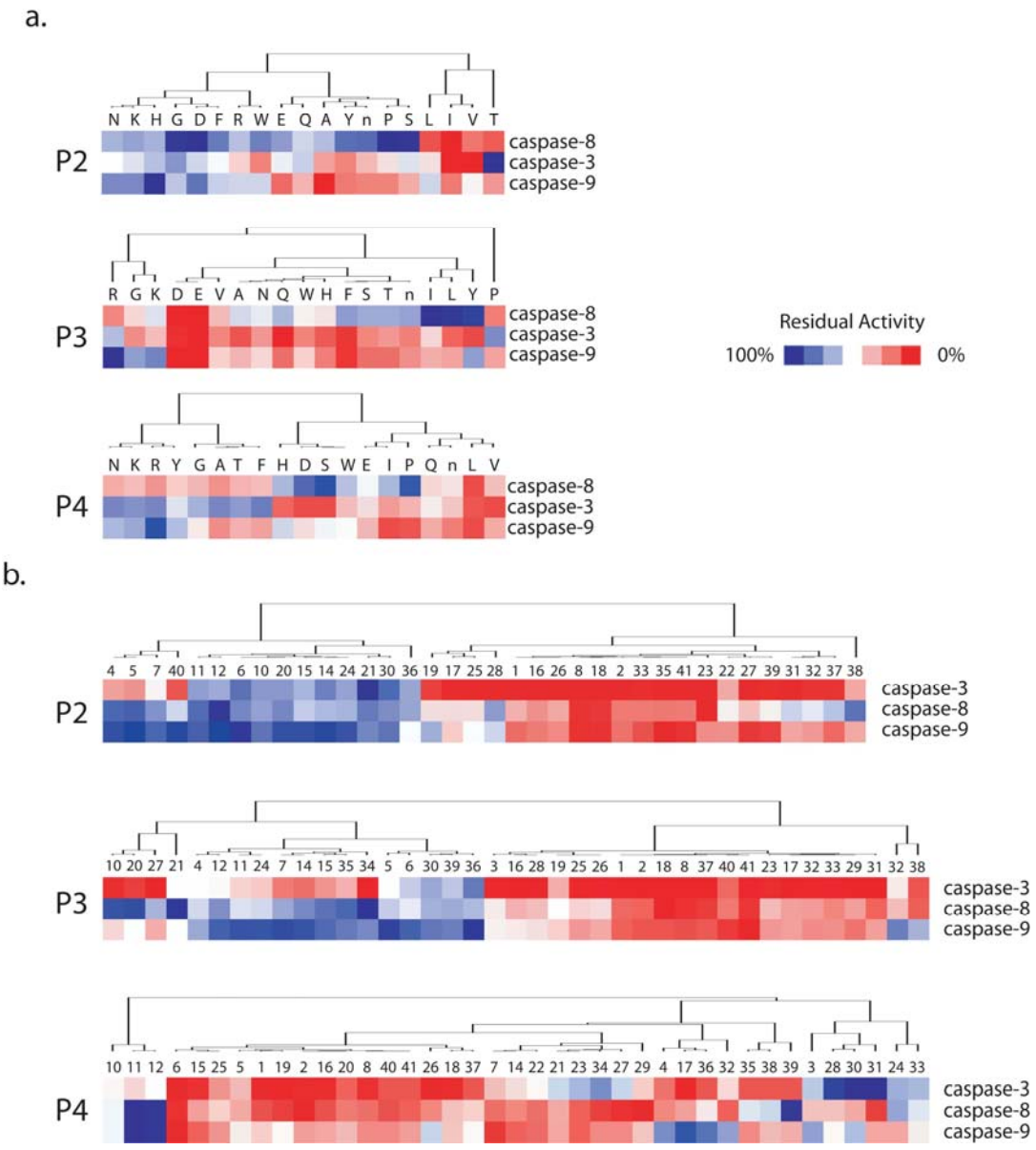


Fig S1. Screening of PSCLs against recombinant caspases-3, 8 and 9. Purified recombinant caspases-3, 8 or 9 were pre-incubated with inhibitor sub-libraries followed by addition of fluorescent substrates. Fluorescence was measured at a set endpoint and residual enzyme activity was calculated from the ratio of normalized fluorescence signal of inhibited and control non-

inhibited samples (see experimental methods). Screening data for peptide libraries in which the constant position contains (a.) natural amino acids and (b.) non-natural amino acids as indicated along the horizontal axis. Cluster diagrams (also called heat maps) were generated using a hierarchical clustering algorithm that converts residual activity values into a color format. Red and blue squares represent 0% and 100% residual activity respectively.

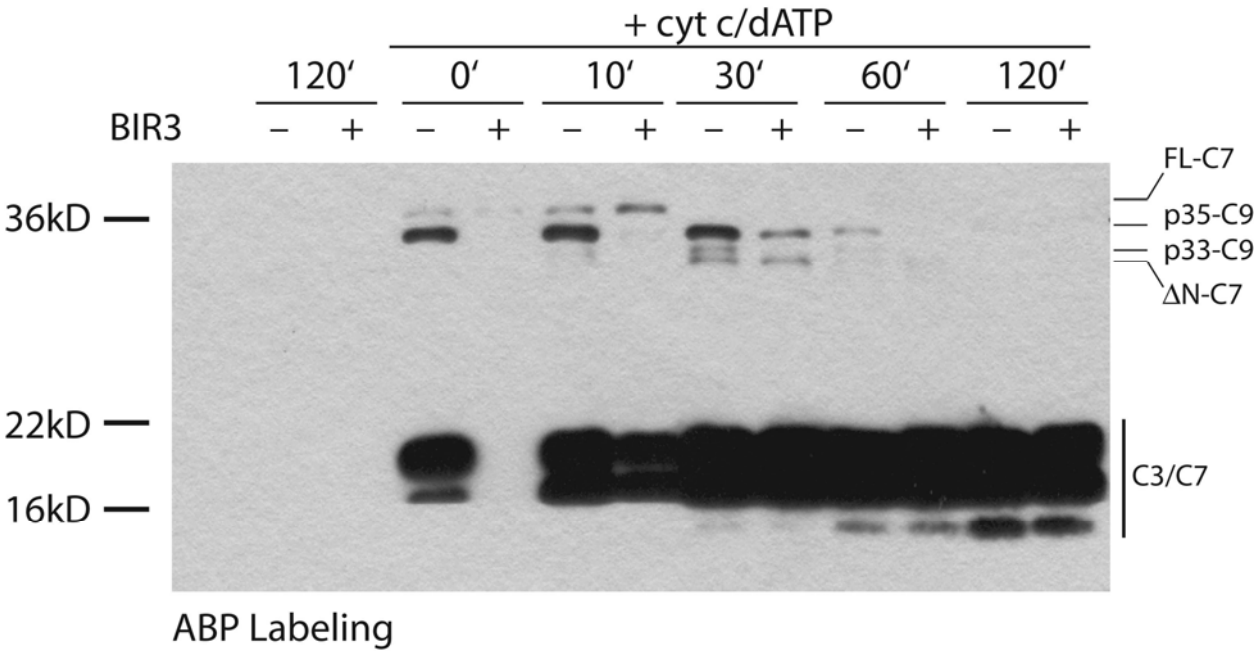


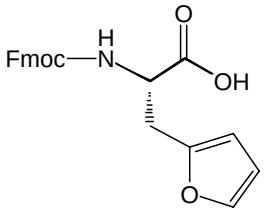
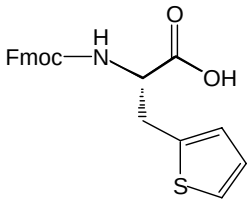
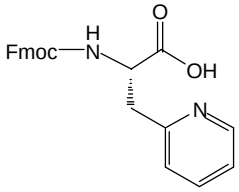
Figure S2. Inhibition of caspase activity by recombinant Bir3 domain. Cytosolic extracts (293) were induced to undergo intrinsic apoptosis by addition of cytochrome c and dATP for the indicated times followed by addition of 1μM Bir3 for 5min. followed by addition ofKMB01 (20 μM) to label residual caspase active sites for an additional 30 min. at 37˚C. Labeled caspase active sites were visualized by SDS-PAGE analysis followed by blotting for biotin using streptavidin-HRP.

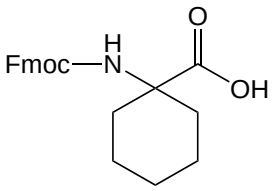
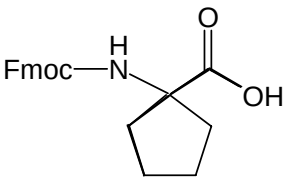
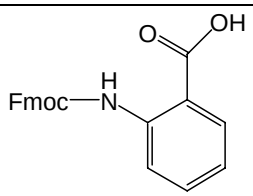
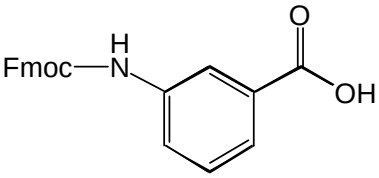
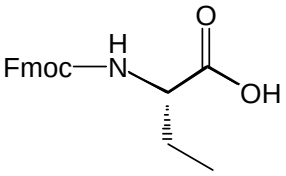
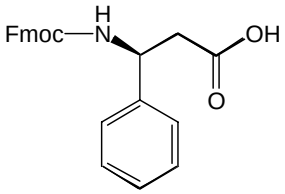
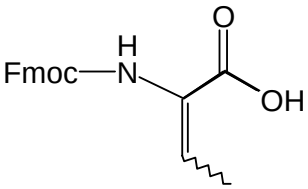
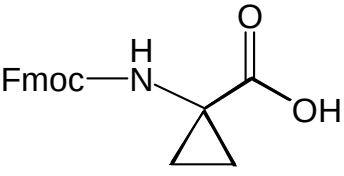
Compound	Specificity Region	Target	caspase-3 Ki(app)	caspase-3 SD	caspase-7 Ki(app)	caspase-7 SD	caspase-8 Ki(app)	caspase-8 SD	caspase-9 Ki(app)	caspase-9 SD
ZVAD-fmk	V-A-D	3,7,8,9	25,922	143	<5,000		203,286	8,499	<5,000	–
KMB01	E-V-D	3,7,8,9	577,913	45,269	288,213	66,292	164,052	3,621	175,210	18,908
AB06	D-3-V-D	3,7	7,456,511	798,842	968,070	68,614	32,909	8,450	NI	–
AB07	L-E-H-D	9	75,295	3,031	10,447	202	506,912	49,101	20,141	4,323
AB08	L-E-T-D	8	127,835	14,476	19,424	537	599,788	92,747	<5,000	–
AB09	D-E-V-D	3,7	10,922,261	1,698,557	1,529,040	77,901	1,077,839	122,447	<5,000	–
AB11	D-E-P-D	3,7,8,9	2,482,333	105,882	199,341	28,649	580,547	20,679	47,362	3,041
AB12	D-29-V-D	3,7	5,652,900	239,568	783,840	33,955	271,626	1,403	NI	–
AB13	D-34-V-D	3,7	3,416,050	659,375	279,519	19,810	<5,000	–	NI	–
AB15	26-34-V-D	3,7	133,705	15,167	ND	–	NI	–	NI	–
AB16	26-3-V-D	3,7	484,495	25,590	24,185	1,904	121,650	28,335	NI	–
AB17	26-E-V-D	3,7	781,733	110,714	448,155	27,317	126,323	21,861	NI	–
AB18	31-E-T-D	8	216,040	3,111	234,945	30,823	572,012	158,775	12,320	3,986
AB19	31-E-23-D	8	179,086	8,237	42,994	1,325	396,225	92,743	NI	–
AB20	29-E-T-D	8	570,900	60,825	181,332	11,206	1,071,401	340,849	41,300	1,038
AB28	6-E-8-D	3,7,8,9	1,020,213	293,979	272,619	34,569	817,077	99,766	300,767	26,860
AB29	D-E-11-D	None	341,429	ND	ND	–	90,503	17,462	NI	–
AB30	D-30-11-D	None	448,179	138,195	ND	–	NI	–	NI	–
AB31	D-30-V-D	None	64,873	12,220	ND	–	NI	–	NI	–
AB38	P-L-A-D	9	46,108	6,799	27,814	2,163	19,676	2,973	18,004	2,820
AB40	I-L-A-D	9	261,470	30,600	11,256	903	35,174	2,790	48,867	5,435
AB41	I-L-38-D	9	1,582,350	84,782	69,317	10,329	49,815	11,370	35,779	2,544
AB42	I-F-P-D	9	892,045	544	42,594	6,225	22,544	2,482	44,709	2,465
bAB06	D-3-V-D	3,7	2,528,900	336,017	412,413	55,719	29,124	6,460	NI	–
bAB13	D-34-V-D	3,7	6,829,900	365,574	456,884	40,740	<1.000		NI	–
bAB19	31-E-23-D	8	192,225	87,193	40,011	5,210	152,956	32,590	NI	–
bAB38	P-L-A-D	9	28,809	2,347	18,685	6,274	24,000	291	39,872	396

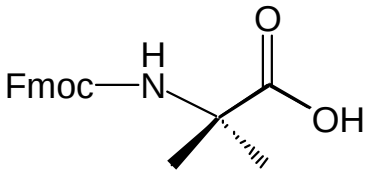
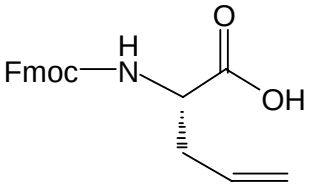
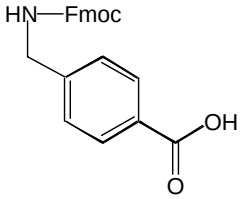
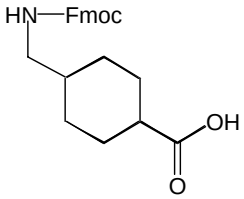
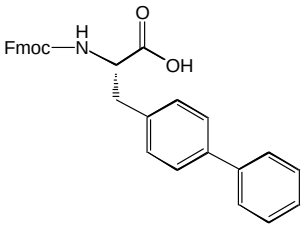
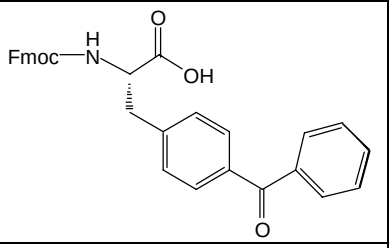
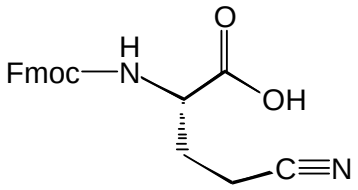
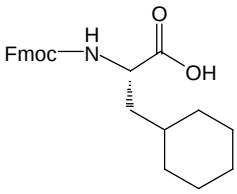
Supplementary Table 1. Ki(app) values for all AB compounds. Ki(app) values (also called Kass or Kobs/I) represent the speed of inhibitor binding to a target enzyme. Units are [M-1s-1]. NI indicates no inhibition at concentrations tested, ND indicates data not determined, SD indicates standard deviation.

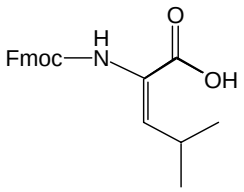
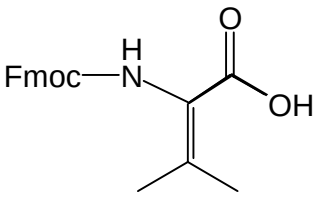
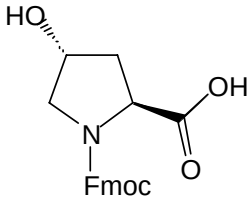
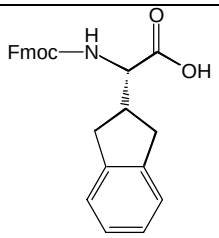
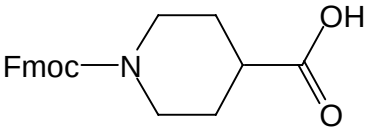
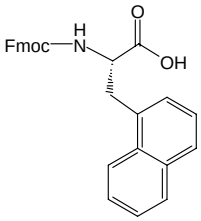
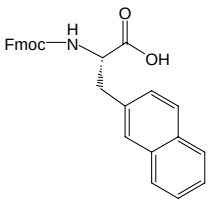
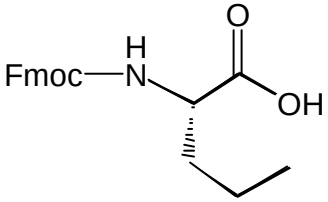
Table S1. Kinetic inhibition values for all caspase inhibitors and probes synthesized based on screening results.

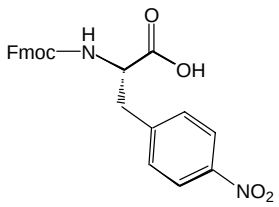
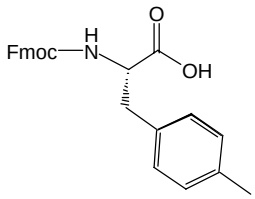
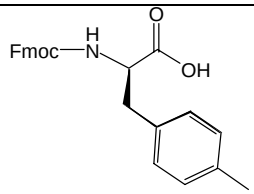
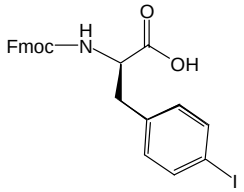
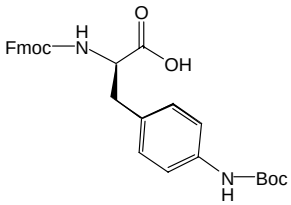
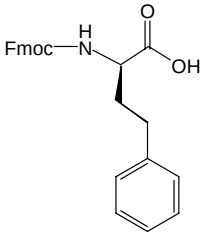
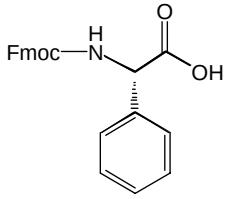
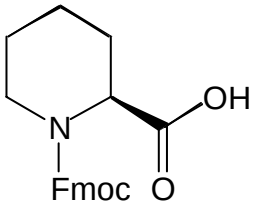
Table S2. NN numbers and structures of non-natural amino acids used as diversity elements in library synthesis. Non-natural amino acids were incorporated into peptides using the commercially available Fmoc-protected amino acids shown in this table.

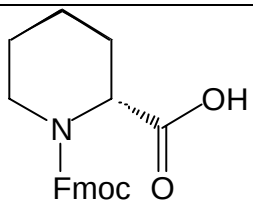
NN #	Amino Acid	Structure
1	(2furyl)alanine	
2	(2thienyl)alanine	
3	2pyridylAla	

4	1amino1cyclohexane carboxylic acid	
5	1amino1cyclopentanecarboxylic acid	
6	2-Abz	
7	3Abz	
8	2Abu	
9	3amino3phenylpropionic acid	
10	dehydroAbu	
11	ACPC	

12	Aib	
13	AllylGly	
14	Amb	
15	Amc	
16	Bip	
17	Bpa	
18	Cba	
19	Cha	

20	deltaLeu	
21	deltaVal	
22	Hyp	
23	Igl	
24	Inp	
25	1-Nal	
26	2-Nal	
27	Nva	

28	4-nitroPhe	
29	4MethylPhe	
30	4Methyl-DPhe	
31	Phe(pI)	
32	Phe4NH(Boc)	
33	hPhe	
34	Phg	
35	pip	



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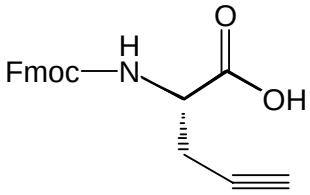
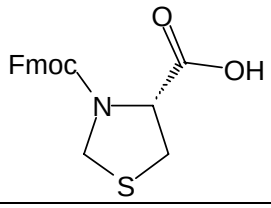
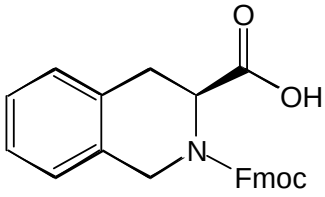
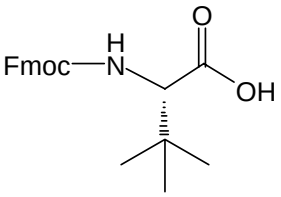
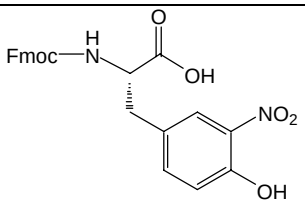
37	propargylglycine	
38	Thz	
39	Tic	
40	Tle	
41	3-NitroTyr	
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